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## DETAILED DESCRIPTION

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[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to the anti-oxidant which makes an active principle the caffeic acid dimer of moss vegetable origin, and the caffeic acid dimer prepared using an enzyme, ferric chloride, or sodium periodate, for example.

[0002]

[Description of the Prior Art]Although oxygen is indispensable to aerobic organisms including human being, it is known that the free radical of the oxygen molecule origin called active oxygen will bring a living body an obstacle. The obstacle of the cell by such active oxygen or a gene is related to generating and advance of cancer, diabetes mellitus, etc. of a lifestyle-related disease, and it is said that it is one of the causes of aging. In the living body, the mechanism which disassembles and stabilizes an oxidant by oxidation repressible enzyme, for example, superoxide dismutase, and catalase exists to various oxidation obstacles produced by the hyperoxidation of lipid. The oxidation inhibitor in the living body is presumed to have played the role important for an oxidation restrained defense mechanism with the biophylaxis mechanism by these enzymes. For example, there is a report of many -- the vitamin E which is a substance of lipophilicity stabilizes a biomembrane physically, or acts as a stop agent of the chain reaction of the free radical in the hyperoxidation process of lipid. These days, search of the natural oxidation inhibitor taken in as a food composition is performed, and many researches are made about the ingredient of vegetable origin, such as epigallocatechin gallate, caffeic acid, etc. which are contained in sesamol of sesame seed origin, sesaminol, and tea catechin. Thus, although it has been shown clearly that the ingredient which controls oxidation exists so much in vegetation, it is not reported at all that a caffeic acid dimer has antioxidation nature.

[0003]Although caffeic acid has a structural formula shown in a figure, Caffeic acid. With the gestalt of 1-(3, 4-dihydroxyphenyl)-6,7-dihydroxy-1,2-dihydro-2,3-naphthalenedicarboxylic acid (DDDN) made into the basic unit, moss vegetation. It exists

so much in some included higher plants. DDDN has two asymmetrical carbon in intramolecular, and it exists as the (-) object or racemate in Tracheophyta. however -- moss -- the (+) object -- or -- Only one side of the (-) object exists. Former. It is 1-(6-carboxy-2-oxo-2H-3-pyranyl)-6,7-dihydroxy-1,2-dihydro-2,3-naphthalenedicarboxylic acid (from AKIUROKOGOKE of Jungermanniales among moss vegetation. Although jamesopyrone was isolated, the aromatic ring of DDDN oxidizes in a biosynthesis and this compound is considered to have become alpha-pyrone ring. From these things, it is thought that moss vegetation biosynthesizes DDDN and jamesopyrone through an enantioselective coupling reaction from caffeic acid unlike the biosynthesis system of lignan in Tracheophyta.

[0004]The 4-hydroxycinnamic acid like the caffeic acid considered to be a precursor of DDDN is abundantly contained for vegetation.

When processing vegetation, a possibility that this reaction may occur in the case of the food manufacturing which makes vegetation the origin can be considered.

Cilliers and Singleton, It has reported that various dimers are formed in the solution to which pH was changed nonenzymatic (J. J.L.Cilliers and V.L.Singleton, J. Agric. Food Chem., 39, 1298-1303-1991). However, there is no example of research which controlled the above reactions intentionally using the enzyme and the oxidizer, and made the dimer generate. A simple compound like caffeic acid is not known about the physiology activity of the dimeric compound dimerized and generated the inside of a plant body, and in foodstuffs, but there is especially about research of the oxidation depressant action of a caffeic acid dimer. [ no ]

[0005]

[Problem(s) to be Solved by the Invention]Therefore, this invention makes it a technical problem to provide the new anti-oxidant which makes a caffeic acid dimer an active principle.

[0006]

[Means for Solving the Problem]As a result of inquiring about a physiological function which a caffeic acid dimer has, this invention persons find out having the oxidation depressant action excellent in a caffeic acid dimer, and came to complete this invention. A caffeic acid dimer used by this invention can be extracted from moss vegetation, or can be prepared using an enzyme, ferric chloride, sodium periodate, etc. That is, after ethyl acetate's extracting after making each reagent react to caffeic acid or caffeic acid methyl ester, and carrying out vacuum concentration of the extract, it dips and refines to a silica gel column. What is necessary is just to change a solvent suitably with the target dimer. Thus, a caffeic acid dimer (L-1, L-4, and L-5) which has a structural formula shown in a figure of not less than 95% of purity can be obtained.

[0007]

[Embodiment of the Invention]The anti-oxidant of this invention makes an active principle the caffeic acid dimer which extracts from moss vegetation, or is produced from caffeic acid by preparing, for example. The anti-oxidant which makes the caffeic acid dimer of this

invention an active principle can be made pharmaceutical preparation, such as a tablet, a tablet, and powder, and can be used. The anti-oxidant which makes the caffeic acid dimer of this invention an active principle can also be added and used for an eating-and-drinking article. The anti-oxidant which makes the caffeic acid dimer of this invention an active principle, Per [ since it has radical-scavenger activity ] adult day By taking in 100micro g-1,000 mg in 1 time or several steps, the oxidative cell damage by active oxygen or peroxy lipid can be prevented or improved, and it is useful. Next, an example is given and the preparing method of the caffeic acid dimer of this invention is explained.

[0008]

[Example 1] (Extraction of the caffeic acid dimer from TOSAKAGOKE) The air-dry matter 1g of a TOSAKAGOKE (*Lophocolea heterophylla*) callus, methanol extraction is carried out and separation refinement of the extract is carried out by opposite phase HPLC (ODS 0.5% HCOOH/20%CH<sub>3</sub>CN-H<sub>2</sub>O) after vacuum concentration -- the caffeic acid dimer (L-1) of 1.3 mg was obtained.

[0009]

[Example 2] (Preparation of the caffeic acid dimer by enzymatic process) Caffeic acid It was made to dissolve in 400 ml of pH 7.5 phosphate buffer solutions so that it may become the concentration of 0.4mM, and 1 mg of horseradish origin peroxidase (Sigma Type 1, product made by Sigma) was added to this. Subsequently, hydrogen peroxide of 0.5mM was added and was made to react for 1 hour. After adding 1N chloride to reaction mixture and making it pH 3 acidity, it moved to the separating funnel and washed repeatedly 3 times by 80 ml of diethylether. Subsequently, the water layer was repeated 3 times and 80 ml of ethyl acetate extracted it. separation refinement of the extract produced by making carry out concentration hardening by drying of the ethyl acetate extract is carried out by opposite phase HPLC (ODS 0.5%HCOOH/20%CH<sub>3</sub>CN-H<sub>2</sub>O) -- the caffeic acid dimer (L-1) of 1.5 mg was obtained.

[0010]

[Example 3] (Preparation of the caffeic acid dimer by enzymatic process) The heating and dissolving of 90 mg of caffeic acid, 570.6 mg of beta-cyclodextrin, and 20 ml of the water were put in and carried out to the Erlenmeyer flask of 100-ml \*\*, and it decompressed and deaerated with the aspirator under ultrasonication. 30% hydrogen-peroxide-solution 60mul and 1 mg of horseradish origin peroxidase (Sigma Type 1, product made by Sigma) are added to this, and it was made to react, stirring at 30 \*\* for 1 hour. pH 2 acidity was used with dilute hydrochloric acid after the reaction, and after 20 ml of ethyl acetate extracted repeatedly 3 times, vacuum concentration of the extract was carried out. Separation refinement of the obtained concentrate was carried out with silica gel column chromatography (chloroform ethyl acetate (1:1)), and a 48.9-mg caffeic acid dimer (L-4) was obtained.

[0011]

[Example 4] (Preparation of the caffeic acid dimer using ferric chloride) The aqueous ferric chloride which dissolved the ferric chloride 6 hydrate 3.2g in 3.2 ml of water was prepared. Next, it adds, and the aforementioned aqueous ferric chloride was stirred for 5 hours, and was made to put 1g of caffeic acid, and 60 ml of acetone into the Erlenmeyer flask of 300ml \*\*, and to react under ice-cooling. Subsequently, water-ethyl acetate (1:1) after removing acetone by an evaporator It distributed by 400 ml. Then, vacuum concentration of the extract obtained by extracting repeatedly 3 times with ethyl acetate was carried out. separation refinement of the concentrate is carried out with silica gel column chromatography (chloroform acetone (1:1)) -- the caffeic acid dimer (L-4) of 120 mg was obtained.

[0012]

[Example 5] (Preparation of the caffeic acid dimer using sodium periodate) After adding and carrying out the heating and dissolving of 180 mg of caffeic acid, and 100 ml of the water to the Erlenmeyer flask of 300-ml \*\* and cooling them radiationally to it, it added and 214.5 mg of sodium periodate was made to react for 10 minutes. After it repeated reaction mixture 3 times and 50 ml of ethyl acetate extracted it, 100 ml of saturation salt solutions washed the ethyl acetate layer. Subsequently, vacuum concentration of the obtained ethyl acetate extract was carried out. Separation refinement of the obtained concentrate was carried out with silica gel column chromatography (chloroform ethyl acetate (1:1)), and an 81-mg caffeic acid dimer (L-4) was obtained.

[0013]

[Example 6] (Preparation of the caffeic acid dimer using sodium periodate) The heating and dissolving of 100 mg of caffeic acid methyl ester and 100 ml of the water were put in and carried out to the eggplant flask of 300-ml \*\*, it decompressed and deaerated with the aspirator under ultrasonication, and, subsequently nitrogen gas was aerated. Next, it added and 107 mg of sodium periodate was made to react for 20 minutes. After it repeated reaction mixture 3 times and 50 ml of ethyl acetate extracted it, 100 ml of saturation salt solutions washed the ethyl acetate layer. Vacuum concentration of the obtained ethyl acetate extract was carried out, separation refinement was carried out with silica gel column chromatography (chloroform ethyl acetate (4:1)), and a 10.4-mg caffeic acid dimer (L-5) was obtained. Next, the example of an examination which checked the effect of this invention is shown.

[0014]

[The example 1 of an examination] (Antioxidation activity of a caffeic acid dimer) The antioxidation activity of the caffeic acid dimer obtained in the example was measured by the method (J. Agric.Food Chem., Vol.35, No.5, p.809-812, 1987) of Osawa and others. That is, an equivalent amount of rabbit stored blood and isotonic solutions (10mM phosphate buffer solution / 152mM NaCl, pH 7.4) were mixed, and 4 \*\*, 1,500 x g (3,500 rpm), and centrifugal separation for 20 minutes were performed 3 times, and were washed. The hypotonic solution (10mM phosphate buffer solution and pH 7.4) was well mixed with the

washed corpuscle, and 4 \*\*, 20,000xg (11,000 rpm), and centrifugal separation for 40 minutes were performed 4 times. Obtained loose precipitation portion (ghost) It used and the antioxidation activity of the caffeic acid dimer was examined. The caffeic acid dimer (L-1, L-4, and L-5) obtained in the example was prepared so that it might be set to 0, 0.01, 0.1, 1, and 10mM by first concentration, and it mixed with the above-mentioned erythrocyte membrane ghost, and oxidized by adding an oxidizer. As contrast, same processing was performed using the vitamin E which is a known anti-oxidant. Subsequently, an absorbance is measured for TBA reaction by a deed and 532 nm, and it is the bottom about a fixed quantity of oxidation products. Absorbance sample additive-free in evaluation of antioxidation activity It carried out by computing the ghost oxidation quotient defined by the following formula from an absorbance when each sample is added by considering it as 100%. Oxidation of the ghost is controlled and it is shown as the thing with this low ghost oxidation quotient that antioxidation activity is high. In spectrometry, it deducted from the absorbance by making into a blank value the absorbance produced by performing TBA reaction by ghost additive-free as contrast. A result is shown in Table 1.

[0015]

[Equation 1] Ghost oxidation quotient (%) = (absorbance / sample additive-free absorbance) x100 [0016] if a caffeic acid dimer (L-1, L-4, and L-5) is added -- concentration -- anacritic antioxidation activity was shown and the activity was higher than caffeic acid. As mentioned above, antioxidation activity (radical-scavenger activity) was observed in the caffeic acid dimer (L-1, L-4, and L-5) by the result of the example of an exam, and it turned out that prevention and an improvement of the oxidative cell damage by active oxygen or peroxy lipid are useful. Since a caffeic acid dimer has the high solubility to water compared with caffeic acid, its fitness in the case of using for a drink etc. is good. The stability at the time of considering it as solution compared with caffeic acid is high, a caffeic acid dimer has the good durability of an effect, and it is useful.

[0017]

[Table 1]

Ghost oxidation quotient (%)

	Sample concentration (mM)	0	0.01	0.1	1	10
-. Vitamin E		100	93	88	74	59
caffeic acid		100	82	87	62	35
caffeic-acid dimer (L-1)	--	100	90	88	50	30
caffeic-acid dimer (L-4)	--	100	90	84	43	18
caffeic-acid dimer (L-5)	--	100	80	87	61	15

[0018] Next, an example explains the example of use of the caffeic acid dimer of this invention.

[0019]

[Example 7] (Manufacture of the drink which blended the anti-oxidant) After mixing a raw material by the combination shown in Table 2, the container was filled up, it heat-sterilized and the drink which gave oxidation depressant action was manufactured.

[0020]

[Table 2]

----- mixed isomerism-ized sugar 15.0 (% of the weight)

Fruit juice 10.0 citrate 0.5 caffeic-acid dimer \* 0.0005 Perfume 0.1 calcium carbonate 0.5  
Water 73.9 ----- \*L-1, L-4, or L-5[0021]

[Example 8] (Manufacture of the tablet which blended the anti-oxidant) After mixing a raw material by the combination shown in Table 3, application-of-pressure molding was carried out and the tablet which gave oxidation depressant action was manufactured.

[0022]

[Table 3]

----- hydrated crystal grape sugar 93.5 (% of the weight)

Caffeic acid dimer \* 0.005 Calcium 5.0 Sugar ester 1.0 Perfume 0.5 -----  
\*L-1, L-4, or L-5[0023]

[Example 9] (Manufacture of the tablet which blended the anti-oxidant) The raw material was mixed by the combination shown in Table 4, and the tablet which gave oxidation depressant action was manufactured.

[0024]

[Table 4]

----- cornstarch 49.15 (% of the weight)

Hydrated crystal grape sugar 47.34 crystalline cellulose 2.5 Carboxymethyl-cellulose  
calcium 0.32 caffeic-acid dimer \* 0.01 Perfume 0.68 ----- \*L-1, L-4, or L-5  
[0025]

[Effect of the Invention]The anti-oxidant which makes the caffeic acid dimer of this invention an active principle can be made pharmaceutical preparation, such as a tablet, a tablet, and powder, and can be used. The anti-oxidant which makes the caffeic acid dimer of this invention an active principle can also be added and used for an eating-and-drinking article. The anti-oxidant which makes the caffeic acid dimer of this invention an active principle, Per [ since it has radical-scavenger activity ] adult day By taking in 100micro g-1,000 mg in 1 time or several steps, the oxidative cell damage by active oxygen or peroxy lipid can be prevented or improved, and it is useful.

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PRIOR ART

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**EFFECT OF THE INVENTION**

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MEANS

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[Example 3] (Preparation of the caffeic acid dimer by enzymatic process) The heating and dissolving of 90 mg of caffeic acid, 570.6 mg of beta-cyclodextrin, and 20 ml of the water were put in and carried out to the Erlenmeyer flask of 100-ml \*\*, and it decompressed and deaerated with the aspirator under ultrasonication. 30% hydrogen-peroxide-solution 60mul and 1 mg of horseradish origin peroxidase (Sigma Type 1, product made by Sigma) are added to this, and it was made to react, stirring at 30 \*\* for 1 hour. pH 2 acidity was used with dilute hydrochloric acid after the reaction, and after 20 ml of ethyl acetate extracted repeatedly 3 times, vacuum concentration of the extract was carried out. Separation refinement of the obtained concentrate was carried out with silica gel column chromatography (chloroform ethyl acetate (1:1)), and a 48.9-mg caffeic acid dimer (L-4) was obtained.

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[0012]

[Example 5] (Preparation of the caffeic acid dimer using sodium periodate) After adding and carrying out the heating and dissolving of 180 mg of caffeic acid, and 100 ml of the water to the Erlenmeyer flask of 300-ml \*\* and cooling them radiationally to it, it added and 214.5 mg of sodium periodate was made to react for 10 minutes. After it repeated reaction mixture 3

times and 50 ml of ethyl acetate extracted it, 100 ml of saturation salt solutions washed the ethyl acetate layer. Subsequently, vacuum concentration of the obtained ethyl acetate extract was carried out. Separation refinement of the obtained concentrate was carried out with silica gel column chromatography (chloroform ethyl acetate (1:1)), and an 81-mg caffeic acid dimer (L-4) was obtained.

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[0014]

[The example 1 of an examination] (Antioxidation activity of a caffeic acid dimer) The antioxidation activity of the caffeic acid dimer obtained in the example was measured by the method (J. Agric.Food Chem., Vol.35, No.5, p.809-812, 1987) of Osawa and others. That is, an equivalent amount of rabbit stored blood and isotonic solutions (10mM phosphate buffer solution / 152mM NaCl, pH 7.4) were mixed, and 4 \*\*, 1,500 x g (3,500 rpm), and centrifugal separation for 20 minutes were performed 3 times, and were washed. The hypotonic solution (10mM phosphate buffer solution and pH 7.4) was well mixed with the washed corpuscle, and 4 \*\*, 20,000xg (11,000 rpm), and centrifugal separation for 40 minutes were performed 4 times. Obtained loose precipitation portion (ghost) It used and the antioxidation activity of the caffeic acid dimer was examined. The caffeic acid dimer (L-1, L-4, and L-5) obtained in the example was prepared so that it might be set to 0, 0.01, 0.1, 1, and 10mM by first concentration, and it mixed with the above-mentioned erythrocyte membrane ghost, and oxidized by adding an oxidizer. As contrast, same processing was performed using the vitamin E which is a known anti-oxidant. Subsequently, an absorbance is measured for TBA reaction by a deed and 532 nm, and it is the bottom about a fixed quantity of oxidation products. Absorbance sample additive-free in evaluation of antioxidation activity It carried out by computing the ghost oxidation quotient defined by the following formula from an absorbance when each sample is added by considering it as 100%. Oxidation of the ghost is controlled and it is shown as the thing with this low ghost oxidation quotient that antioxidation activity is high. In spectrometry, it deducted from the absorbance by making into a blank value the absorbance produced by performing TBA reaction by ghost additive-free as contrast. A result is shown in Table 1.

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[0017]

[Table 1]

Ghost oxidation quotient (%)

----- Sample concentration (mM) 0 0.01 0.1 1 10. -----  
 --. Vitamin E 100 93 88 74 59. caffeic acid 100 82 87 62 35 caffeic-acid dimer (L-1) -- 100  
 90 88 50 30 caffeic-acid dimer (L-4) -- 100 90 84 43 18 caffeic-acid dimer (L-5) -- 100 80 87  
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 caffeic acid dimer of this invention.

[0019]

[Example 7] (Manufacture of the drink which blended the anti-oxidant) After mixing a raw material by the combination shown in Table 2, the container was filled up, it heat-sterilized and the drink which gave oxidation depressant action was manufactured.

[0020]

[Table 2]

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 Fruit juice 10.0 citrate 0.5 caffeic-acid dimer \* 0.0005 Perfume 0.1 calcium carbonate 0.5  
 Water 73.9 ----- \*L-1, L-4, or L-5[0021]

[Example 8] (Manufacture of the tablet which blended the anti-oxidant) After mixing a raw material by the combination shown in Table 3, application-of-pressure molding was carried out and the tablet which gave oxidation depressant action was manufactured.

[0022]

[Table 3]

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[0024]

[Table 4]

----- cornstarch 49.15 (% of the weight)  
Hydrated crystal grape sugar 47.34 crystalline cellulose 2.5 Carboxymethyl-cellulose  
calcium 0.32 caffeic-acid dimer \* 0.01 Perfume 0.68 ----- \*L-1, L-4, or L-5

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- 3.In the drawings, any words are not translated.

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CLAIMS

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[Claim(s)]

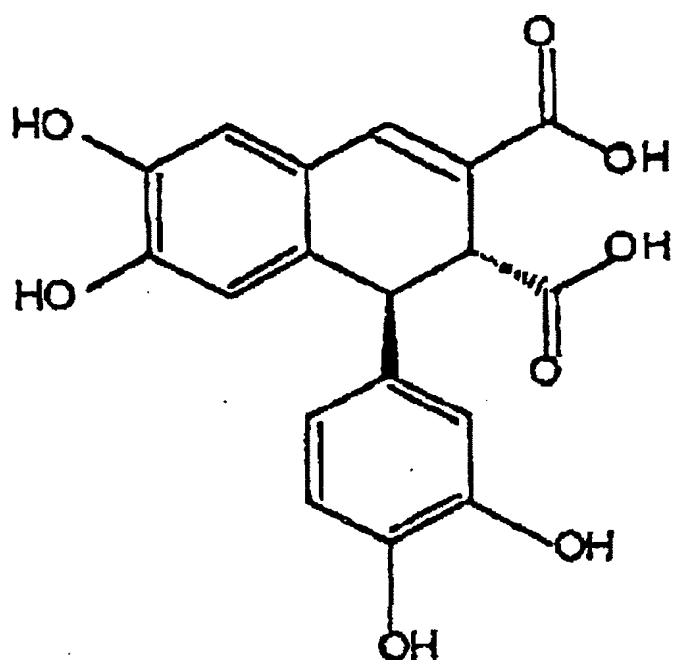
[Claim 1]An anti-oxidant which makes a caffeic acid dimer an active principle.

[Claim 2]The anti-oxidant according to claim 1 whose caffeic acid dimer is of moss vegetable origin.

[Claim 3]The anti-oxidant according to claim 1 in which a caffeic acid dimer is prepared using an enzyme, ferric chloride, or sodium periodate.

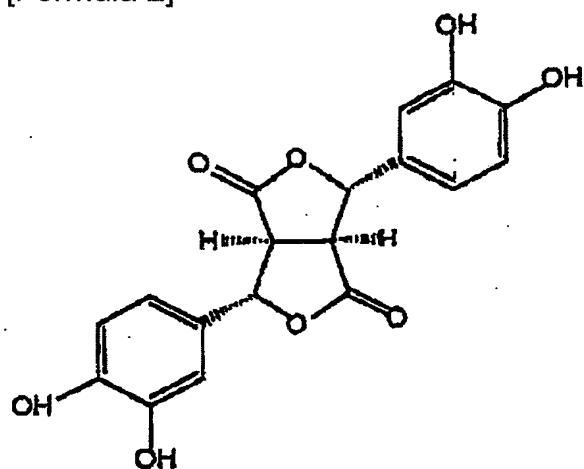
[Claim 4]The anti-oxidant according to claim 1, wherein caffeic acid dimers are a compound shown with the chemical formula 1, the chemical formula 2, or the chemical formula 3, and/or its salts.

[Formula 1]



コーヒ一酸二量体 (L-1)

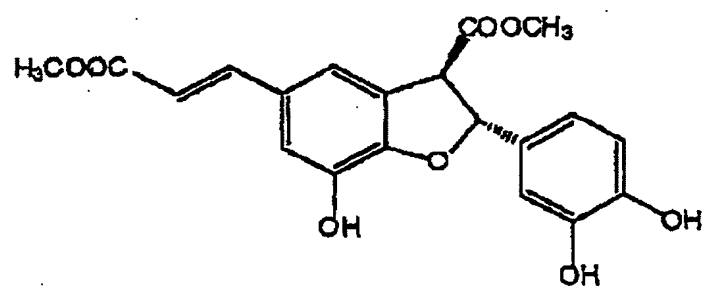
[Formula 2]



コーヒ一酸二量体 (L-4)

[Formula 3]





コーヒー酸二量体 (L-5)

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[Translation done.]